White, Brown, and Beige; Type 2 Immunity Gets Hot

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The biogenesis of beige fat is poorly understood. In recent issues of *Nature* and *Cell*, Brestoff et al. (2014) and Lee et al. (2015) demonstrate that resident innate lymphoid cells in subcutaneous fat generate and activate beige adipocytes, producing thermogenesis.

The current obesity epidemic has focused a great deal of interest on the control of energy homeostasis and the ensuing adaptive changes that result in obesity's many complications. Much of the attention has centered on adipose tissue, which can undergo remodeling in response to nutrient status and ambient temperature. Adipose tissues (with their constituent cells-adipocytes) come in different colors that reflect unique metabolic roles. In the fed state, adipocytes in white fat depots accumulate triglycerides. Upon exposure to cold, adipocytes in brown fat depots respond to cold-induced activation of the sympathetic nervous system by increasing lipolysis and upregulating the expression and activity of the uncoupling protein UCP1 to stimulate thermogenesis. And recent studies suggest the presence of a third type of fat cell, termed "beige," a brown-like adipocyte that is present mainly in subcutaneous white fat depots, derived from unique precursor cells, and can be induced by various stimuli to also generate heat (Wu et al., 2013).

The activities of these different fat-cell types adapt to conditions of metabolic stress like obesity. Both white and brown adipocytes accumulate excessive lipid in states of overnutrition, and the thermogenic activity of beige fat appears to be decreased, while resistance to both insulin and catecholamines develops in all adipocytes in the obese state. One intrinsic feature of the obese state that might link these outcomes is activation of the innate immune system; numerous studies suggest that obesity-generated inflammation is strongly associated with, and perhaps causal to, insulin resistance and type 2 diabetes. This state of low grade, sustained inflammation is accompanied by macrophage switching in adipose tissue from a type 2 (or M2) polarization state generally associated with tissue surveillance and increased insulin sensitivity, to a type 1 (or M1) polarization state characterized by secretion of proinflammatory cytokines that attenuate insulin action (Lumeng and Saltiel, 2011). Moreover, alterations in adipose tissue macrophage content and polarization state appear to occur downstream of a coordinated inflammatory circuit that includes crosstalk between infiltrating T cells with a T helper 1 (Th1) cell pattern of activation, and reduced Th2 cell responses, with contributions from B cells, NK cells, NKT cells, eosinophils, neutrophils, and mast cells (Schipper et al., 2012). Thus, the relative reduction in type 2 immunity might parallel or even generate increased proinflammatory macrophage polarization in obesity, which in turn influences adipocyte function and response to hormones.

Two recent studies have shed light on a new player in adipocyte regulation interleukin-33 (IL-33), a member of the interleukin 1 family of cytokines known to regulate group 2 innate lymphoid cells (ILC2s). Previous studies had shown that IL-33 administration induces production of Th2 cell-associated cytokines such as IL-5, IL-13, and IL-10 and leads to generation of an M2 phenotype of macrophages in adipose tissue, along with reduced adiposity and insulin resistance (Miller et al., 2010). Brestoff et al., (2014) and Lee et al. (2015) now clarify the mechanisms by which ILC2s appear to exert their interesting effects and suggest further that these effects are critically dependent on the browning of white fat. Chawla's laboratory had previously hinted that M2 macrophages and type 2 immune signaling pathways contribute to the browning of subcutaneous white fat (Nguyen et al., 2011; Qiu et al., 2014). They proposed a process in which cold activates eosinophils, the main source of IL-4, which in turn directs a type 2 immune response circuit to recruit M2 macrophages and induce tyrosine hydroxylase expression in these cells to synthesize catecholamines. Catecholamines such as norepinephrine activate β-adrenergic receptors in adipocytes to turn on the thermogenic program, including induction of UCP-1. Lee et al. (2015) now report that activation of ILC2s is upstream of this process; in addition to changes in macrophage function, the IL-4 derived from these cells also stimulates the proliferation of bipotential adipocyte precursors (APs) within subcutaneous fat and enhances their differentiation into beige adipocytes. ILC2s had previously been identified within lymphoid structures in mouse and human mesenteric adipose tissues and known to sustain visceral adipose tissue eosinophils and M2 macrophages that function to improve insulin sensitivity (Molofsky et al., 2013).

Because ILC2s are sensitive to IL-33, Lee et al. (2015) administered IL-33 to mice adapted to thermoneutrality to activate ILC2s in subcutaneous white fat. Interestingly, IL-33 treatment induced the browning or beiging of subcutaneous fat, increased UCP1 expression, and promoted energy expenditure under thermal stress. They noted that, at the same time, IL-33 promoted proliferation of bipotential platelet-derived growth factor receptor α^+ (PDGFRα^+) APs. They demonstrated that PDGFRα^+ APs express IL-4 receptor, and ILC2s promoted proliferation of PDGFRα^+ APs in an IL-4 and/or IL-13-dependent manner. More importantly, experiments in mice lacking ILC2s showed that these cells were required for proliferation of PDGFRα^+ APs in response to IL-33 treatment. Interestingly, proliferation of PDGFRα^+ APs was age-dependent, because 5-week-old mice

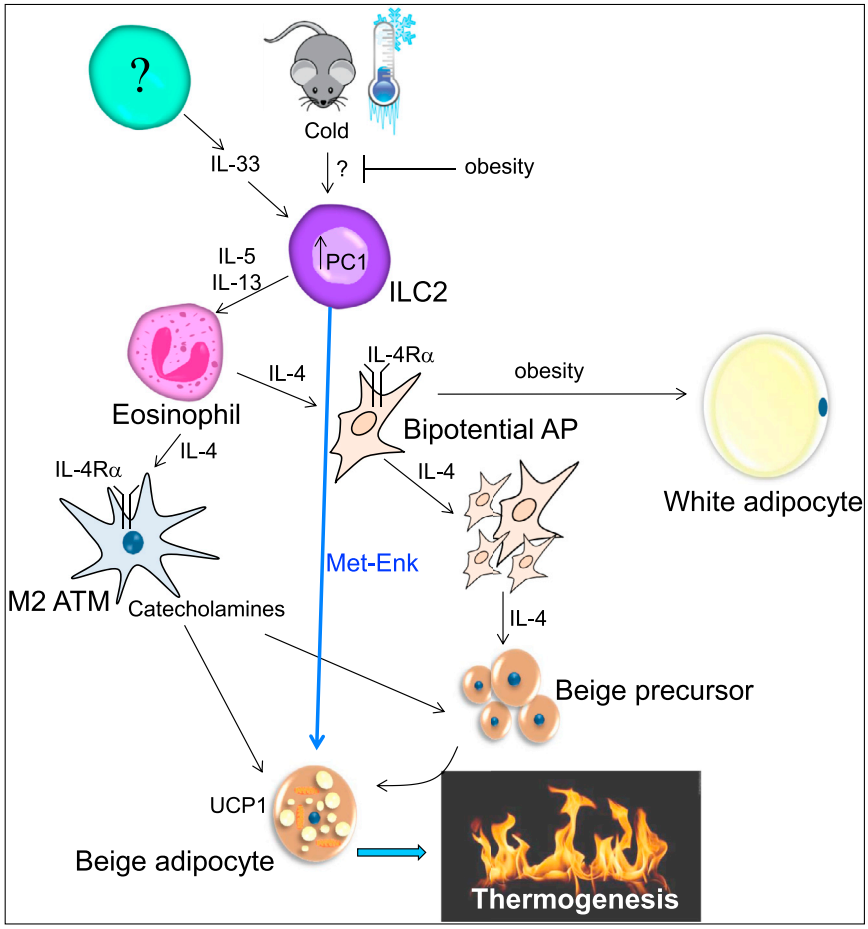

Figure 1. Activation of Type 2 Innate Lymphoid Cells Induces Beige Fat Development in White Adipose Tissue

Although how cold might trigger the pathway is obscure, activation of ILC2s with IL-33 induces beige fat biogenesis. Once activated, ILC2s secrete IL-5 and/or IL-13 to sustain and stimulate adipose tissue eosinophils and M2 macrophages. Eosinophils produce IL-4, which directly acts on bipotential adipocyte precursor (AP) cells through IL-4 receptor α (IL-4Rα). This leads to the proliferation and commitment of $Myf5^{-}PDGFR\alpha^{+}$ APs to beige adipogenic precursors. These cells eventually differentiate into mature beige adipocyte with increased amounts of UCP1. IL-4 also activates M2 polarized adipose tissue macrophages (M2 ATM) to produce catecholamines that induce and activate beige fat via the beta-adrenergic receptor pathway. Obesity reduces the activation of ILC2s, and APs are thus induced to differentiate into white adipocytes for storage of excess energy. ILC2s are also reported to generate methionine-enkephalin (MetEnk) in response to IL-33 via induction of the prohormone convertase (PC1). MetEnk can act directly on beige adipocytes to increase UCP1 and thermogenesis.

showed the most robust proliferation of $PDGFR\alpha^{+}$ APs. While IL-4 signaling occurred in both myeloid cells and $PDGFR\alpha^{+}$ APs, IL-4 was sufficient to expand the pool of $PDGFR\alpha^{+}$ APs in a cell-autonomous manner in subcutaneous fat.

$PDGFR\alpha^{+}$ APs are bipotential cells that can differentiate into either beige or white adipocytes (Lee et al., 2012). To address whether type 2 cytokine signaling also directs the commitment of $PDGFR\alpha^{+}$ APs, they showed that treatment of purified $PDGFR\alpha^{+}$ APs derived from subcutaneous fat in vitro with IL-4 induced beige adipocyte markers, whereas mice with loss of type 2 cytokine signaling demonstrated impaired expression of beige precursor cell markers in $PDGFR\alpha^{+}$ APs and decreased capacity for beige adipogenesis in vivo. Taken together, Lee et al. (2015) propose that ILC2s coordinate a complex circuit of pathways involving the generation of IL-4 through eosinophils that controls recruitment of specific precursors for beige adipogenesis, as well as the generation from M2 macrophages of catecholamines that acutely stimulate these newly produced beige adipocytes for thermogenesis.

Brestoff et al. (2014) independently demonstrated this unique role for IL-33 and ILC2s in the beiging of subcutaneous white adipose tissue, but focus on a different mechanism. These investigators showed that IL-33-deficient mice developed metabolic abnormalities while on normal diet, suggesting that this cytokine plays a key role in maintaining metabolic health and insulin sensitivity. They also identified IL-33-responsive ILC2s in human white fat and showed that activation was decreased in human obesity, consistent with previous findings on macrophage polarization (Lumeng and Saltiel, 2011). IL-33 increased thermogenesis and energy expenditure in obese mice, but in contrast to the report by Lee et al. (2015), the beneficial effects of the cytokine were identical in eosinophil-deficient mice, suggesting that IL-33 induces beiging independently of eosinophils and IL-4. In order to elucidate the mechanism of the biological effect of IL-33, they employed whole-genome expression profiling of ILC2s and found prohormone convertase 1 (PC1) to be significantly enriched in ILC2s after treatment with IL-33. PC1 is an endopeptidase that converts peptide hormones into their active form, and additional studies revealed that ILC2s generated methionine-enkephalin (MetEnk) in response to IL-33. Further experiments revealed that MetEnk directly stimulated UCP1 expression in isolated beige adipocytes and induced browning of white fat. This is surprising, because this peptide binds to delta- and mu-opioid receptors, known to couple to the heterotrimeric G protein Gi, and thus reduce cAMP levels in cells, an effect not expected to increase UCP1 induction or energy expenditure (Law et al., 2000). However, perhaps other pathways or cascades are involved unique to beige adipocytes.

Notwithstanding these questions, together these papers suggest that biological regulation of ILC2s with IL-33 administration and activation of a type 2 cytokine signaling network might provide new insights into how mammals regulate thermogenic capacity and adapt to environmental stresses like overnutrition and cold (Figure 1). Aside from resolving the issue of the requirement for eosinophil function in controlling browning of white fat by ILC2s, there are numerous

other questions that arise from this work. First, what is the true physiological role of type 2 immunity in subcutaneous fat function? Is IL-33 required or sufficient for cold adaptation? How do IL-33-deficient mice respond to high-fat diet? IL-33 is produced from a variety of cells, many of which are found in adipose tissue. What might trigger its release? Is there a regulatory pathway involved in its synthesis or secretion that is somehow induced by cold? How is the pathway downregulated during obesity? Is there a role for activation of the sympathetic nervous system in this process? Among the most interesting questions are the therapeutic implications of this work in patients with obesity and type 2 diabetes, focusing on these two new peptides. IL-33 had beneficial effects in these studies on energy expenditure, and might be cardioprotective (Sanada et al., 2007), but is also known to promote eosinophilia and allergic inflammation (Molofsky et al., 2013). Met-Enk is a delta-opioid agonist with a long history of target discovery for analgesia, depression, and ischemic preconditioning, although concerns with side effects have limited enthusiasm. Also confusing is the potential mechanism by which this peptide might exert its effects on fat cells. Thus, it will be crucial to answer these questions and elucidate the relative importance of these pathways compared to others that increase energy expenditure. In the meantime, it is becoming increasingly evident that studies at the intersection of type 2 immunity and energy metabolism are really heating up.

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